

In vitro calcium imaging

Qiping Dong Qiang Chang

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An abbreviated version of this protocol was published in eLIFE in Mar 2018

Mechanism and consequence of abnormal calcium homeostasis in Rett syndrome astrocytes

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Detailed protocol

In vitro calcium imaging

Cells:

human iPSC derived astrocytes: 7 days after the start of terminal differentiation;
mouse primary hippocampal astrocytes: 4 weeks in vitro.

Solution:

artificial cerebrospinal fluid (aCSF, in mM): 120 NaCl, 3 KCl, 15 HEPES, 1 MgCl₂, 2 CaCl₂, 20 Glucose (pH7.4).

aCSF with 0 mM Ca²⁺, 2 mM EGTA and 1 μM thapsigargin (TG) was used to deplete the ER Ca²⁺ load.

Preparation for imaging:

For the human iPSC derived astrocytes and some mouse primary astrocytes, intracellular Ca²⁺ was indicated by Fluo-4 or Rhod-2. Cells were bulk-loaded for 15 min at 37°C in aCSF containing Fluo-4/AM (12.5 μg/ml), pluronic acid (0.05%), and DMSO (0.1%).

For mouse primary astrocytes from GCaMP6s mice, cells were treated with 4-OH tamoxifen (0.5 μM, Sigma-Aldrich Inc.) for 24 hr to induce the expression of GCaMP6s.

Calcium imaging:

The cells were transferred to a chamber, then calcium imaging was performed using a Nikon A1 confocal microscope at room temperature. All image data were taken in the frame-scanning mode at 1 frame every 2 s (in the experiments using GCaMP6s as the Ca²⁺ indicator, 1 frame every 1 s). Fluo-4 or GCaMP6s was excited at 488 nm. For store-operated Ca²⁺ entry (SOCE), normal aCSF was perfused after TG induced ER Ca²⁺ depletion.

Data analysis:

The raw ND2 files were loaded into memory using Python. The metadata (including the frame rate, the pixel size, etc.) was read using python-bioformats (<https://pythonhosted.org/python-bioformats/>) and the imaging data was read into multidimensional arrays using Numpy (<https://numpy.org/>).

Ca²⁺ signals were presented as relative fluorescence changes ($\Delta F/F_0$) from specified regions of interest (ROIs). ROIs were selected using an automated segmentation algorithm (see the source code), while somata and processes were manually identified on the basis of morphology. The spontaneous Ca²⁺ elevations from the soma and process of astrocytes were analyzed separately. For the analysis of Ca²⁺ imaging data in acute mouse brain slices, only Ca²⁺ elevations in soma and major process of astrocytes were included.

For the traces with baseline drift, baseline correction was performed (see the source code).

The peaks were detected using the algorithm developed by Matlab (findpeaks function). Calcium elevation events were detected with thresholds of 3 times of standard deviation of the baseline. The frequency, amplitude, and the full-width at half maximum (FWHM) were calculated and measured (see the source code).

Source code:

ROI identification:

https://github.com/qdong8/calcium_data_analysis/blob/main/segment.py

Baseline correction, event detection, frequency, amplitude, and the full-width at half maximum (FWHM) calculation:

https://github.com/qdong8/calcium_data_analysis/blob/main/ca_signal.py

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

- Dong, Q. and Chang, Q. (2020). In vitro calcium imaging. Bio-protocol Preprint. bio-protocol.org/prep723.
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